

Research report

Consistent magnetic-field induced dynamical changes in rabbit brain activity detected by recurrence quantification analysis

Andrew A. Marino^{a,*}, Erik Nilsen^{a,b}, Clifton Frilot^b

^aDepartment of Orthopaedic Surgery, Louisiana State University Health Sciences Center, 1501 Kings Highway, P.O. Box 33932, Shreveport, LA 71130-3932, USA

^bCollege of Engineering Sciences, Louisiana Tech University, Ruston, LA 71270, USA

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Abstract

The reports dealing with the effects of electromagnetic fields (EMFs) on brain electrical activity have been inconsistent. We suspected that the use of linear methods and models accounted for some of the variability, and we explored the issue by using a novel approach to study the effects of EMFs on the electroencephalogram (EEG) from rabbits. The EEG was embedded in phase space and local recurrence plots were calculated and quantified to permit comparisons between exposed and control epochs from individual animals. Statistically significant alterations in brain activity were observed in each animal ($n=10$) when it was exposed to 2.5 G, 60 Hz, as assessed using each of two recurrence-plot quantifiers. Each result was replicated; a positive-control procedure ruled out the possibility that the effect of the field was a product of the method of analysis. Measurements performed while the rabbits were under anesthesia suggested that the effect was mediated by *N*-methyl-D-aspartate and/or α_2 -adrenoceptors. No differences were found between exposed and control epochs in any animal when the experiment was repeated after the rabbits had been killed, indicating that a putative interaction between the field and the EEG electrodes could not account for the observed effects. We conclude that EMF transduction resulting in changes in brain electrical activity could be demonstrated consistently using a nonlinear method of analysis.

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1. Introduction

Low-frequency electromagnetic fields (EMFs) are common in the environment. Their effects on brain electrical activity have been studied in human and animal experiments, typically by means of spectral analysis, but the reports have not revealed a recognizable pattern of response to fields. Exposure to 16 Hz, 289 mG altered activity in rat hippocampal slices [12], but neither a zero frequency field that was 5000 times stronger nor a 4-Hz pulsed microwave field had any effect on the electroencephalogram (EEG) from human subjects [15,24]. Exposure to 200–300 mG, 60 Hz, produced both positive and

negative effects, depending on the strength of an accompanying electric field [10,11]. In a series of animal and human studies, we showed that weak magnetic fields of 0–60 Hz altered the EEG in roughly half the subjects studied [2–6,17]. In this study we addressed ourselves generally to the question of why the effects of fields on brain activity have appeared to be variable.

Recent evidence suggested that the baseline EEG is at least partly generated by low-dimensional chaotic sources [1,14,20,23]. These reports raised the possibility that the effects of fields on the EEG might involve nonlinear neuronal networks. If so, approaches specifically geared toward quantifying dynamical activity in the EEG would be expected to be more sensitive than spectral analysis for detecting changes due to the fields, and consequently might yield more consistent results. Recurrence quantification analysis (RQA) is a method of analyzing time-series

*Corresponding author. Tel.: +1-318-675-6180; fax: +1-318-675-6186.

E-mail address: amarino@lsuhsc.edu (A.A. Marino).

data that does not parse the activity into linear and nonlinear categories [25,27,28]. Rather, it quantifies the activity according to objective rules irrespective of the number or dynamical nature of the individual sources, or how their outputs might combine to produce the measured signal.

We modeled the effect of the field on the basis of a complexity conjecture (explained below), and used RQA to test the hypothesis that the field altered the dynamical activity of the brain in individual animals. The data strongly supported the hypothesis, and showed that the effect of the field depended on consciousness.

2. Materials and methods

2.1. Exposure system

Magnetic fields were produced using a coaxial arrangement of four square coils [19], each 66 cm on a side and wound using 12-gauge magnet wire (Fig. 1). Each coil was dipped in epoxy to minimize potential vibrations from interactions between the coil turns, and wrapped with grounded aluminum foil to eliminate possible effects due to electric fields. The number of turns in the outer and inner coil pairs (85 and 35, respectively) and the coil spacings (± 33.4 cm and ± 8.5 cm from the center-line) were chosen so that the magnetic field strength varied by less than 5% throughout the volume occupied by the rabbit, as predicted using commercial software (MF3D, ERM Inc., Pittsburgh, PA); the homogeneity was verified

by direct measurement using a three-axis magnetic field sensor (Bartington MAG-03, GMW, Redwood City, CA).

The exposure units were energized by power supplies consisting of an isolation transformer, autotransformer, and series capacitors, and were operated in series resonance at 60 Hz to eliminate powerline harmonics. Fourier analysis of the coil currents showed that the strongest harmonics were more than 40 dB below the fundamental. The power supplies were housed in solid copper boxes to minimize the magnetic fields created by surface eddy currents.

During an experiment, the rabbit was restrained in an acrylic box mounted inside a light-tight wooden box that minimized environmental influences and standardized the animal's sensory environment. The assembly was placed in the four-coil unit such that the rostral–caudal and unit axes were parallel, and the rabbit was located within the homogeneous region of the field. A field of 2.5 G, 60 Hz was used because it is representative of the largest field typically found in the environment due to the electrical power system. The field was a subliminal stimulus as judged by the complete absence of a behavioral response when the field was presented, and its presentation was not accompanied by any sensory cues to the rabbit. The average geomagnetic field at the location of the rabbit was 305 mG, 22.6° below the horizontal. The geomagnetic component along the direction of the 60-Hz field was 239 mG.

2.2. Animals

Five female (nos. 1–5) and five male (nos. 6–10) New Zealand rabbits were used in the study. All animal

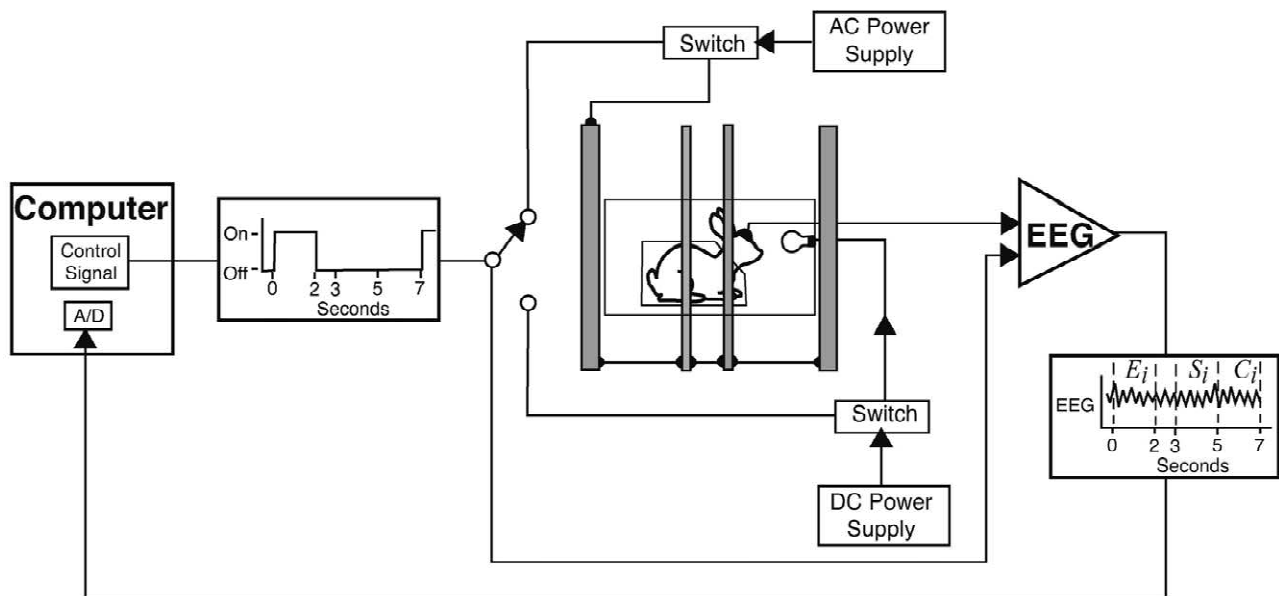


Fig. 1. Schematic representation of the experimental system. A computer-generated timing signal controlled switching of the stimulus. The timing signal was also fed into one of the channels of the EEG amplifier to facilitate identification of the exposed (E), sham (S), and control (C) epochs of the EEG in each trial (the i th trial is illustrated). The location of the rabbit relative to the field-producing coils (shaded bars) is shown.

procedures were approved by the institutional Animal Care and Use Committee. The EEG was recorded over the occipital region, which was under the easily palpable suture of the parietal and interparietal cranial bones. The indifferent and ground electrodes were respectively 2.5 cm and 5 cm more rostral. The electrodes (0.5 cm in diameter) were attached to the shaved scalp using conducting paste (EC2, Grass, Quincy, MA); the impedance (1–3 k Ω) was measured before and after each experiment (EZM 5, Grass).

The EEG was measured using an amplifier capable of resolving a source voltage of 0.1 μ V (Model 4400, Nihon Kohden, Irvine, CA). The signal was filtered to pass 0.3–35 Hz, amplified, digitized at 512 Hz (12-bit), and stored on a hard drive. Switching in or out the 60-Hz notch filter on the amplifier had no effect on the recorded signal. Any 60-Hz energy in the recorded signal was more than 40 dB below the maximum Fourier component of the EEG.

Where explicitly indicated, experiments were performed on rabbits anesthetized using ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg), both given intramuscularly. The rabbits were killed by intravenous injection of pentabartibol (75 mg/kg).

2.3. Procedure

Independent experiments were performed on each rabbit to allow a determination of each animal's ability to detect the stimulus. Presentation of the magnetic field commenced 5 min after the rabbit was placed in the light-tight box. A trial consisted in the application of the field for 2 s (*E* epoch), followed by a field-free period of 5 s; a minimum of 60 trials were run. The procedure was then repeated, using light as the stimulus. For this purpose, a weak red light source (approximately 50 lumens at the corneal surface of the eye) was mounted inside the light-tight box. The rise-times of the currents through the coils and the light source were approximately 1 μ s. All experi-

ments were replicated at least once; the time between successive experiments on the same animal varied from 30 min to several weeks.

The EEG was measured continuously while the rabbit was in the light-tight box. The signal from the last 2 s of each trial was used as the control (*C* epoch) for the corresponding *E* epoch. The signal from the 2 s preceding the *C* epoch was defined as the sham (*S* epoch); it was also analyzed statistically relative to *C* to evaluate the possibility that any positive results might be attributable solely to our analytical method. After the rabbits were killed, the field was applied as previously, and voltage measurements were made from the scalp electrodes to evaluate the possibility of passive field interactions with the electrodes.

2.4. Analysis

The complexity conjecture (Fig. 2) formed the basis of our approach. The baseline EEG was regarded as a combination of contributions from different brain regions, and the determinism in the combined signal was characterized using RQA. The conjecture that the field caused a change in the EEG by altering one or more of its sources was tested by comparing RQA quantifiers measured in the presence and the absence of the field. Other investigators studied the effects of EMFs on the EEG, and some reported positive effects [2–6,10,12,16,24]. Our method differed from those used previously principally in that it was designed to capture any structure that might exist in the EEG, not simply linear structure.

Trials containing movement artifacts were removed from the recorded voltage. The remaining trials constituted a scalar time series, *S*, consisting of voltages at discrete times $t = 1, 2, 3 \dots N$. *S* was embedded in a five-dimensional state space using a time delay of 1 [8]. Each point in phase space represents state vector (**X**) of the system [13]; in our case, **X** consisted of five sequential values from the time series. The resulting trajectory, which described the

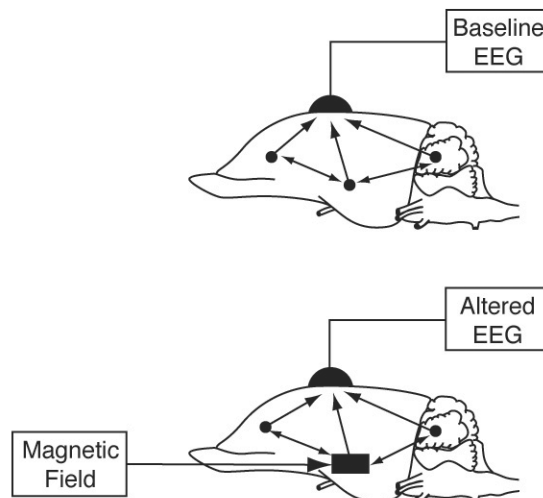


Fig. 2. The complexity conjecture. The baseline EEG is viewed as a complex combination of signals from many regions of the brain. The combined signal, as characterized by RQA, is altered as a consequence of field transduction.

evolution of \mathbf{X} , was used to produce a 2-dimensional plot of points that each corresponded to a pair of state vectors that were near one another ('recurrence plot') [7]. A point was plotted at the location addressed by (i,j) whenever \mathbf{X}_j was near \mathbf{X}_i . Two states were defined as near only if both were contained within a five-dimensional sphere having a radius less than 15% of the minimum radius such that all points were near.

The recurrence plot was quantified using percent recurrence (%R) and percent determinism (%D), defined respectively as the number of recurrent points divided by the possible number of recurrent points, and the number of recurrent points located on lines parallel to the main diagonal of the diagram divided by the number of recurrent points [25,27,28]. Recurrence plots can reveal patterns that cannot be detected by eye. However, quantitative descriptors (%D and %R are only two examples) do not provide direct insight into the physiological basis of the activity that produced the aspect of the pattern captured by the descriptors. Calculation of %R and %D was carried out using software provided by Webber [26]; the parameters radius and line were set to 15 and 2, respectively.

For statistical evaluation of the results of each experiment, the first five trials were discarded and the next 50 artifact-free trials were used to compare the values of the nonlinear quantifiers, using the Wilcoxon signed-rank test. The data are presented in terms of the mean \pm S.D. of the quantifiers, and the mean \pm 95% confidence limits of the Wilcoxon metric

$$\left[\sum_{i=1}^{50} 2(E_i - C_i)^2 / (\bar{E} + \bar{C}) \right]^{1/2}$$

where E_i and C_i are respectively the parameter values in the exposed and control epochs, \bar{E} and \bar{C} are the corresponding epoch means. The quantifiers were regarded as independent planned comparisons, and therefore no corrections were made for multiple tests. $P < 0.05$ was interpreted to indicate a significant difference.

In preliminary studies involving only rabbit no. 1 we followed an iterative procedure to determine the portion of the E epoch that contained the effect of the field. The location thus determined was then studied prospectively in the remaining nine rabbits.

The power spectrum of the rabbit EEG was described previously [6].

3. Results

The complexity conjecture and RQA were applied to the EEG data from rabbit no. 1 to ascertain the conditions that maximized discrimination between the E and C epochs. First, the entire EEG was unfolded in phase space, and %D as a function of time was calculated at minimum resolution (80 time-series points) for each trial. When the results were averaged across all the trials, we found an apparent time-dependent increase in %D which occurred early in the E epoch (Fig. 3). To optimize our ability to detect an effect, corresponding portions of the signal in the E and C epochs were systematically compared using the Wilcoxon signed-rank test to evaluate E versus C , and S versus C . The purpose of the procedure was to identify the portion of the signal that was most responsive to the field. We found that 250 ms portion of the signal ('window') centered at 250 ms after commencement of field application yielded the lowest P s for E versus C when $P > 0.05$ for S versus C . The result for %D was $37.1 \pm 2.7\%$ for the E segments (centered at 250 ms, width of 250 ms), compared with $13.3 \pm 2.2\%$ for the controls (5.25 sec, 250 ms) ($P < 0.05$); the %D in the sham segments, $14.1 \pm 2\%$, did not differ from the controls. The %R values were $10.1 \pm 0.5\%$, $2.7 \pm 0.7\%$, $2.6 \pm 0.6\%$, for the exposed, control, and sham segments, respectively. The recurrence plots from which %D and %R were calculated (Fig. 4) clearly revealed that a quantitative difference had occurred.

The window width and location developed for rabbit no. 1 were applied prospectively to four additional female

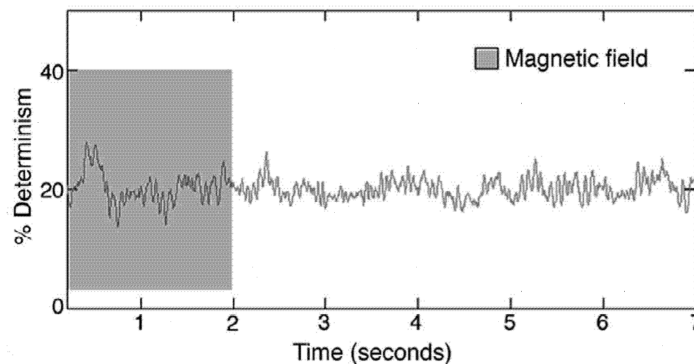


Fig. 3. Percent Determinism in the EEG of rabbit no. 1, averaged over 50 trials.

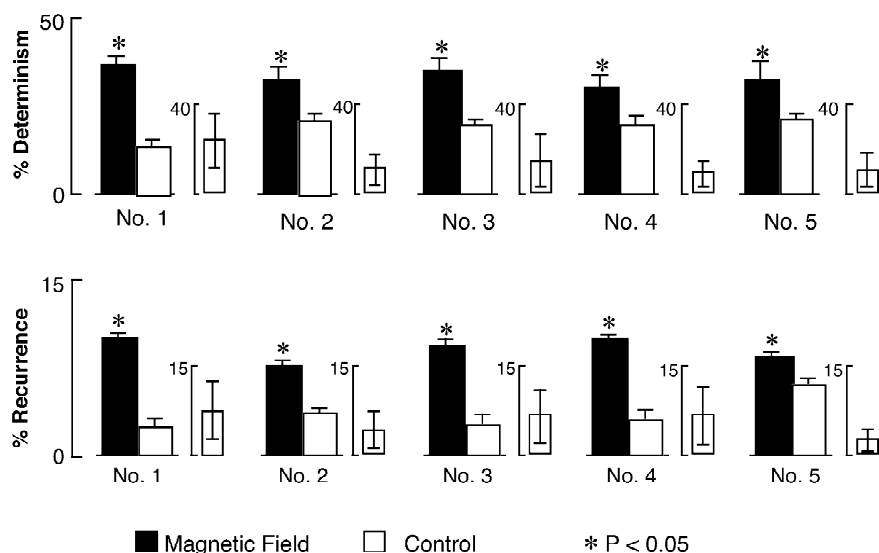


Fig. 4. Local recurrence plots from rabbit no. 1 obtained by concatenating the field (left) and corresponding control (right) segments. The recurrent points are shown as regions of increased density that occur symmetrically about the diagonal. N, point index number.

rabbits, and statistically significant differences in %D and %R were found in all animals tested (Fig. 5). There were no cases of a false positive result, as assessed by comparing the sham and control segments (data not shown).

The effect of light on the EEG was analyzed similarly to the effect of the field, and we found an induced change in %D in rabbit no. 1 (Fig. 6). Again, the local recurrence plots clearly revealed the increased structure induced by the stimulus (Fig. 7). The optimal parameters (determined as previously) were a width of 266 ms centered at 175 ms. When the parameters were applied prospectively to four other female rabbits, statistically significant differences in %D and %R were found in all animals tested (Fig. 8). There were no cases of a false positive result, as assessed by comparing the sham and control segments (data not shown).

We evaluated the reproducibility of the effects of the field by repeating the experiment three additional times for each rabbit, utilizing the window parameters determined initially. In each experiment, %D and %R were statistically significantly greater during the field epochs, compared with the corresponding control epochs, and there were no cases of a false positive result when the sham and control epochs were compared. The results from all four replicate experiments on rabbit no. 1 are shown in Fig. 9.

The experiments were repeated using five male rabbits, employing the same window parameters used to evaluate the effects on the EEG in the females. Again, exposure to 2.5 G, 60 Hz significantly increased %D and %R (Fig. 10), and the results were reproducible when the experiments were repeated (data not shown). The effect of light on the EEG was identical to that found previously for the female

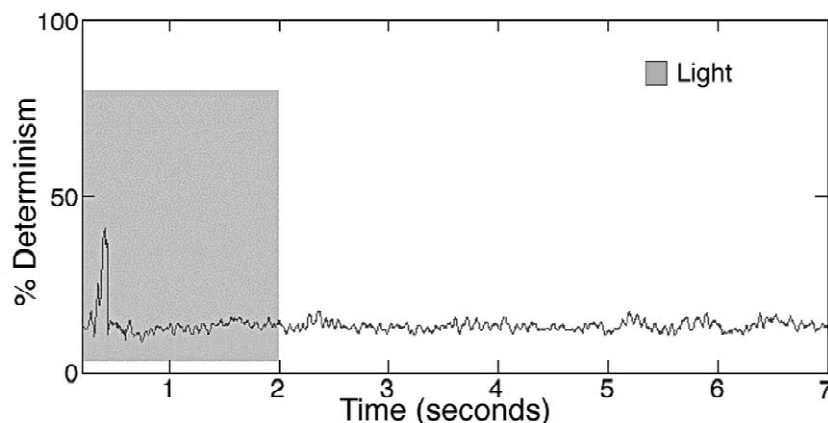


Fig. 5. Effect of 2.5 G, 60 Hz in five female rabbits, as assessed using two RQA quantifiers. For each rabbit and each quantifier, the difference between the exposed and control EEG epochs was evaluated using the Wilcoxon signed-rank test. EEG window centered at 250 ms, with width of 250 ms. The average values of the quantifiers (\pm S.D.) and the 95% confidence limits of the test metric are presented for each rabbit.

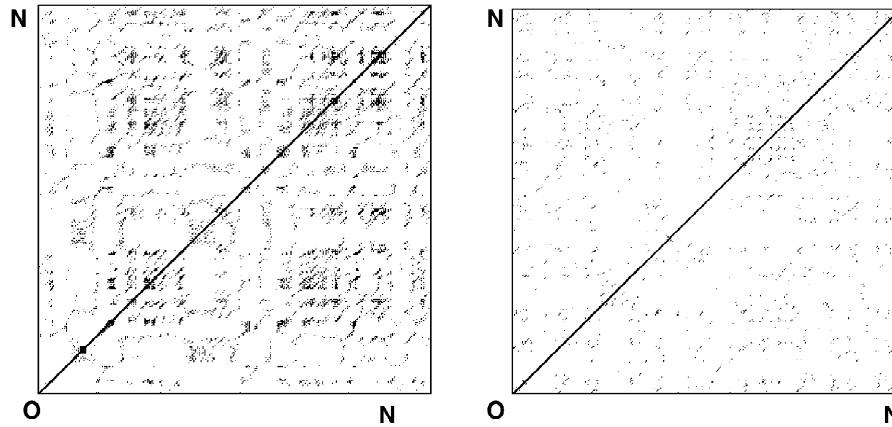


Fig. 6. Percent Determinism in the EEG of rabbit no. 1, averaged over 50 trials.

rabbits (data not shown). In all experiments with the field and light, there were no cases of a false positive result in the comparisons of the *S* and *C* epochs.

To study the effect of the level of consciousness on the ability of the magnetic field to affect brain electrical activity, the experiments were repeated following the induction of anesthesia. The previously observed effect of the field on the EEG was absent in both the female (data not shown) and male (Fig. 11) rabbits. In contrast, anesthesia had no effect on the EEG changes caused by light (Fig. 12).

After the animals were killed and cessation of heart activity was verified, the field experiments were repeated. The input signal to the amplifier was analyzed as previously, and we found that the RQA parameters were essentially zero, and independent of the presence of the field (Fig. 13).

4. Discussion

The magnetic field consistently changed the evolution of the state vector computed from the EEG, as assessed by comparing the *E* and *C* epochs using the RQA parameters %D and %R (Figs. 5 and 10). No false positive comparisons were found when the same statistical procedures were used to compare *S* and *C* epochs, indicating that neither our analytical method nor nonstationarity in the EEG could explain our results.

Several lines of evidence indicated that the altered signal reflected a true physiological response, and not solely a physical effect due to the interaction of the field with the electrodes. First, any physical effect would have been expected to begin at $t=0$, because the rise-time of the current that produced the magnetic fields was nil. The observation that the response commenced 125 ms later was

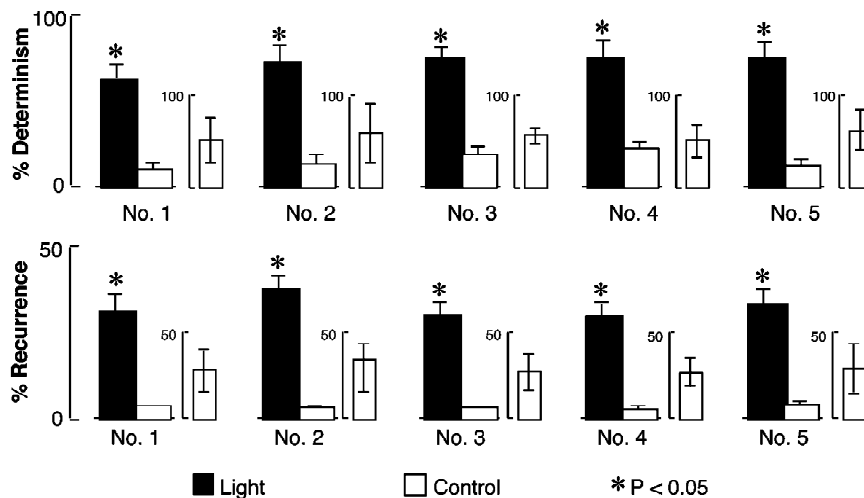


Fig. 7. Local recurrence plots from rabbit no. 1 obtained by concatenating the light (left) and corresponding control (right) segments. The recurrent points are shown as regions of increased density that occur symmetrically about the diagonal. N, point index number.

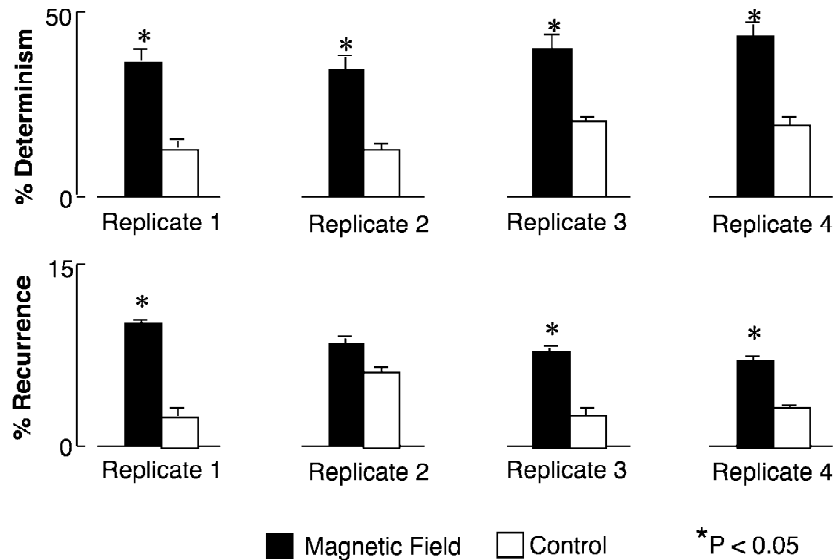


Fig. 8. Effect of light on the EEG in five female rabbits, as assessed using two RQA quantifiers. For each rabbit and each quantifier, the difference between the exposed and control EEG epochs was evaluated using the Wilcoxon signed-rank test. EEG window centered at 175 ms, with width of 266 ms. The average values and 95% confidence limits of the test metric are presented for each rabbit.

consistent with the occurrence of a delay resulting from the biological processes of transduction and propagation of the concomitant electrical change to the measuring electrode. Second, any physical effect would have been present when the measurements were made after the rabbits had been killed. However, we observed essentially no change in %D or %R in the signal measured from the dead rabbit (Fig. 13). Third, the results obtained with the field and with the light were qualitatively similar (Figs. 5 and 10, compared with Figs. 8 and 11). Because of the vast difference in frequency between the two stimuli, the similarity was better explained by assuming that the changes were both

physiological, rather than by assuming that the two different stimuli produced the same kind of physical interaction with the electrodes.

We conclude, therefore, that the field was transduced, resulting in a change in brain electrical activity. A significant aspect of this result is that the effect was seen in essentially every experiment (four replicates in each of ten rabbits). The reproducibility and consistency of these results far exceeded those of any previously reported study involving the biological effects of electromagnetic fields. This consistency was directly attributable to our use of a nonlinear approach, thereby providing what we think is the

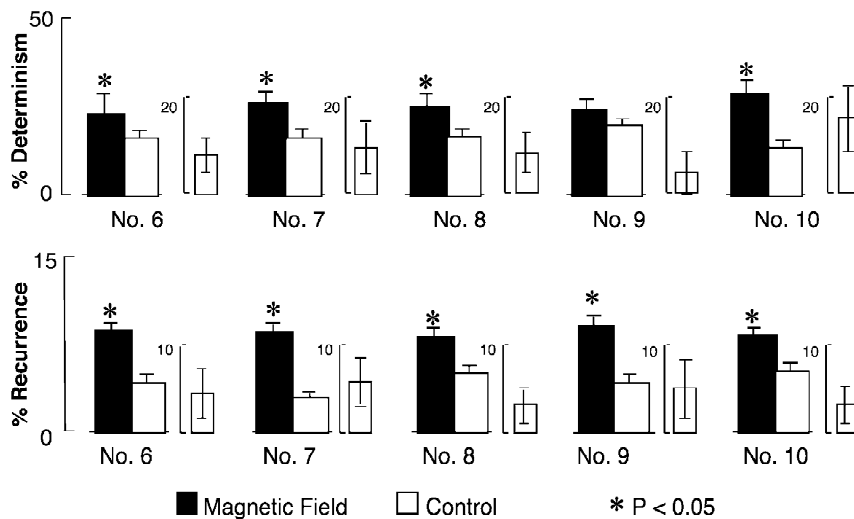


Fig. 9. Reproducibility of the effect of 2.5 G, 60 Hz on two RQA quantifiers of brain electrical activity in rabbit no. 1. EEG window centered at 250 ms, with width of 250 ms. The average values of the quantifiers (\pm S.D.) and the 95% confidence limits of the test metric are presented for each rabbit.

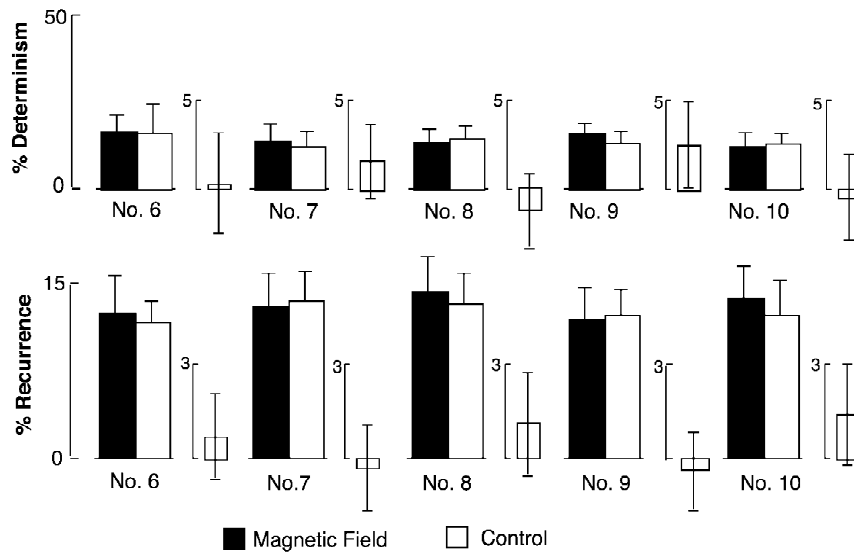


Fig. 10. Effect of 2.5 G, 60 Hz on the EEG in five male rabbits, as assessed using two RQA quantifiers. For each rabbit and each quantifier, the difference between the exposed and control EEG epochs was evaluated using the Wilcoxon signed-rank test. EEG window centered at 250 ms, with width of 250 ms. The average values of the quantifiers (\pm S.D.) and the 95% confidence limits of the test metric are presented for each rabbit.

strongest evidence yet obtained indicating that at least some effects of EMFs on living systems are nonlinear in nature.

Utilization of a nonlinear model (whether characterized with RQA or other methods) is inherently an approach that regards the individual nonlinear system rather than the average behavior of a group, as the proper focus of the analysis. In this study, the field (and the light) caused increased %D and %R in each rabbit. Consequently, both the occurrence of a response and the pattern among individuals in the group were consistent. However, it could just as well been the case that the direction of field-induced

change varied from animal to animal. We have already presented indirect evidence that such was the case with regard to how fields affect the immune system of mice [18]. If the RQA results had been inconsistent (increased %D and %R in some animals, decreased in others), then an attempt to characterize group behavior would have led to averaging away the real effects produced by the stimuli. This consideration suggests that, in principle, statistical analysis of nonlinear dynamical activity ought to be based on individual rather than group behavior. Otherwise, real but inconsistent effects would be hopelessly confounded with the situation where no effects occurred.

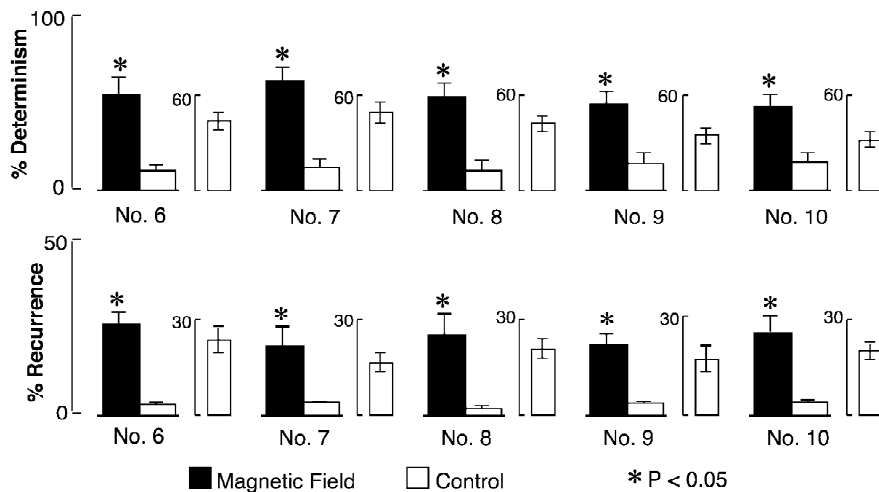


Fig. 11. Effect of 2.5 G, 60 Hz on the EEG in five anesthetized male rabbits, as assessed using two RQA quantifiers. For each rabbit and each quantifier, the difference between the exposed and control EEG epochs was evaluated using the Wilcoxon signed-rank test. EEG window centered at 250 ms, with width of 250 ms. The average value and 95% confidence limits of the test metric are presented for each rabbit.

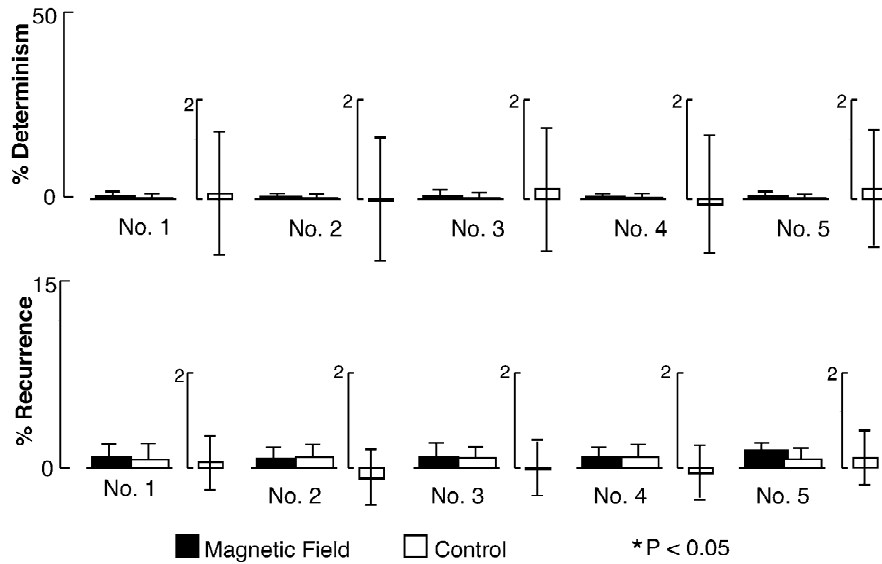


Fig. 12. Effect of light on the EEG in five anesthetized male rabbits, as assessed using two RQA quantifiers. For each rabbit and each quantifier, the difference between the exposed and control EEG epochs was evaluated using the Wilcoxon signed-rank test. EEG window centered at 250 ms, with width of 250 ms. The average values of the quantifiers (\pm S.D.) and the 95% confidence limits of the test metric are presented for each rabbit.

The effect of the field was vitiated by anesthesia with ketamine hydrochloride, an NMDA receptor antagonist, and xylazine, which stimulates α_2 -adrenoceptors [9,21] (Fig. 11). This result suggests that one or both receptors may have mediated signal transduction or signal processing caused by the field. This interpretation is consistent with our observation that the effect of light was not altered by the anesthesia (Fig. 12), because the visual pathway in the rabbit apparently does not involve these receptors, as

judged by the absence of an effect of ketamine hydrochloride and xylazine on the visual evoked potential [22].

Acknowledgements

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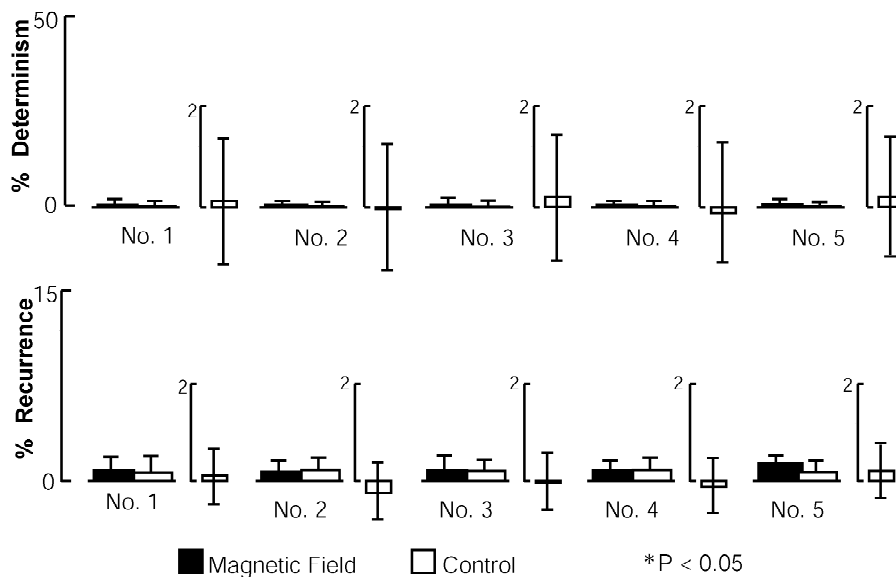


Fig. 13. Effect of 2.5 G, 60 Hz on two RQA quantifiers of the EEG from five female rabbits, assessed after the rabbits had been killed. For each rabbit and each quantifier, the difference between the exposed and control EEG epochs was evaluated using the Wilcoxon signed-rank test. EEG window centered at 250 ms, with width of 250 ms. The average values of the quantifiers (\pm S.D.) and the 95% confidence limits of the test metric are presented for each rabbit.

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